Using molecular genetics to gain insight into allopatric and sympatric speciation of topshells and their parasitic trematodes

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Extended Abstract

1. INTRODUCTION

Prevailing theory suggests that many parasite species have evolved in tight congruence with their hosts, with the parasite phylogeny mirroring that of the host. This theory is based largely on studies of host-parasite interactions between species where strong links would be expected. For example, the highly congruent evolutionary trees of procellariform seabirds and their feather lice (Paterson et al., 1993) are expected as oceanic seabirds breed in large, often monospecific, colonies and lice do not survive long away from their host. Consequently, in this case, there are few opportunities for host switching. Our research employs molecular techniques to test the theory of tight host-parasite congruence in a host-parasite system where high host specificity is not necessarily expected; *Trochoidea* (topshell snails) and digenean trematodes (flatworms). Due to the fine-scale sympatry of the topshell hosts and the complex life cycle of digeneans, the parasites are likely to encounter a range of potential hosts, allowing ample opportunity for host-switching. Topshells belonging to the genera *Melagraphia* and *Diloma* are ubiquitous in the New Zealand intertidal zone, with seven species currently recognised. Six of these species are endemic, the exception being *D. nigerrima*, which also occurs in Chile. Related topshells are found in other parts of the Pacific, most notably along the southern coast of Australia, where seven species are classified as belonging to the genus *Austrocochlea*. Despite different topshell species having slightly different ecological requirements, species may exist in sympatry e.g. during a preliminary study at Purakaunui Inlet, near Dunedin, six species were found within a 20 m radius. *Melagraphia*, *Diloma* and *Austrocochlea* are all potential first-intermediate hosts of digenean trematodes, which infest the snail's digestive gland. During their life cycle digeneans usually parasitise three hosts. The second-intermediate and definitive hosts of these digeneans are unknown, but are likely to be a crustacean and a fish, respectively. Digenean eggs are shed into the water column in the faeces of the definitive host and so, due to the sympatric nature of topshell distribution, developing larvae are likely to encounter a number of potential host species and opportunities for host-switching are great. Topshell phylogeny is currently unresolved, with poorly defined generic boundaries and digenean phylogeny is even less well understood; digeneans which parasitise *Melagraphia*, *Diloma* and *Austrocochlea* consist of a single morphotype which has tentatively been placed in Opecoelidae (Clark, 1958; Miller and Poulin, 2001). Species assignments and phylogenies of both topshells and their digeneans trematodes are being resolved using DNA sequence data. Questions concerning the dispersal, biogeography and evolution of both host and parasite are being addressed by analysing this molecular data in conjunction with data on their geographic distributions. Ultimately the goal of this research is to answer questions about the co-evolution of topshells and their parasites, such as: are the two trees congruent? is there evidence of host-switching and host-addition?

2. METHODS

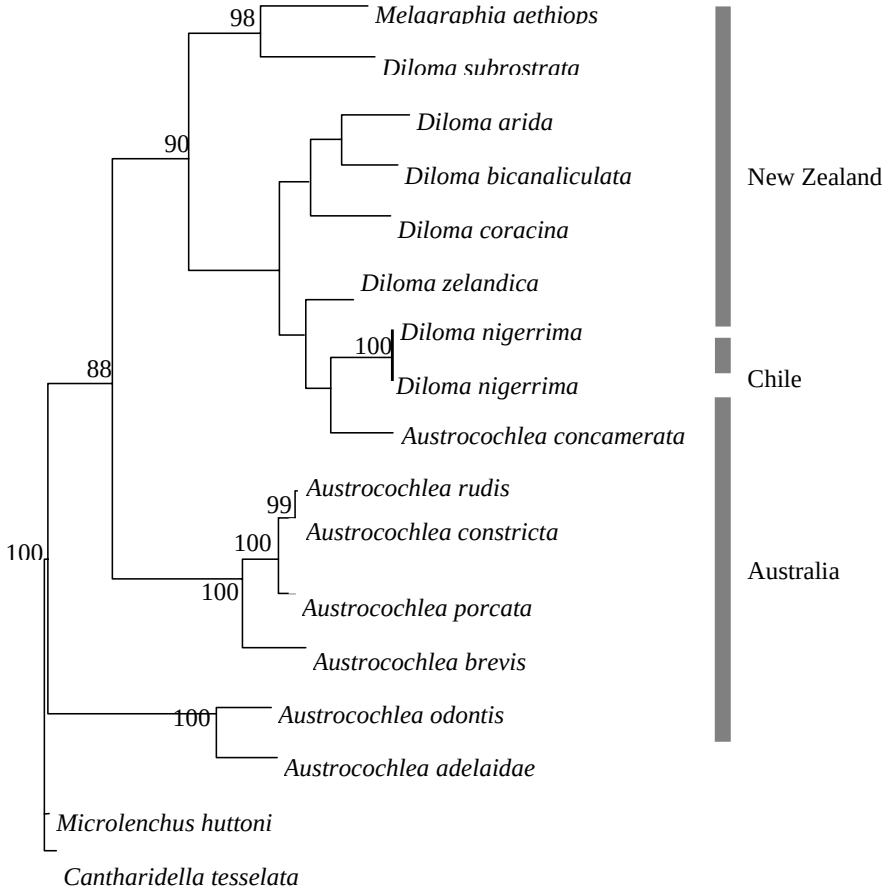
An extensive field study was performed in which >15000 individual topshells from 73 populations (representing 3 genera and 14 species) were collected from beaches in New Zealand, Australia and Chile. Snails were assayed for the presence of digenean parasites. At least 10 snails from each population and all digeneans found were preserved in 70% ethanol for subsequent DNA analysis. DNA was extracted from both topshells and digeneans using a Chelex method (Walsh et al., 1991). Portions of four snail genes (16S, cytochrome oxidase I, 12S and

actin) representing ~2 kilobases (kb) and two digenean genes (16S and ITS2), representing 0.75 kb were sequenced. Resulting sequences were aligned using the ClustalX alignment program (Thompson et al., 1997) and phylogenetic analyses were performed with PAUP* version 4b1.0 software (Swofford, 2002).

3. RESULTS

The molecular phylogeny constructed from the topshell DNA sequence (figure 1) revealed that the majority of the Australian *Austrocochlea* species were basal to New Zealand *Melagraphia* and *Diloma* species. This indicated that these topshells originally dispersed from Australia to New Zealand. The exception is *A. concamerata*, which showed more similarity, over 2 kb sequence, to New Zealand *Diloma* species than other *Austrocochlea* species, suggesting that the presence of *A. concamerata* in Australia resulted from a back dispersal from New Zealand. *D. nigerrima* collected in New Zealand and Chile are indistinguishable at the genetic level indicating that the Chilean species has been correctly assigned and occurs in Chile as the result of a recent dispersal event from New Zealand.

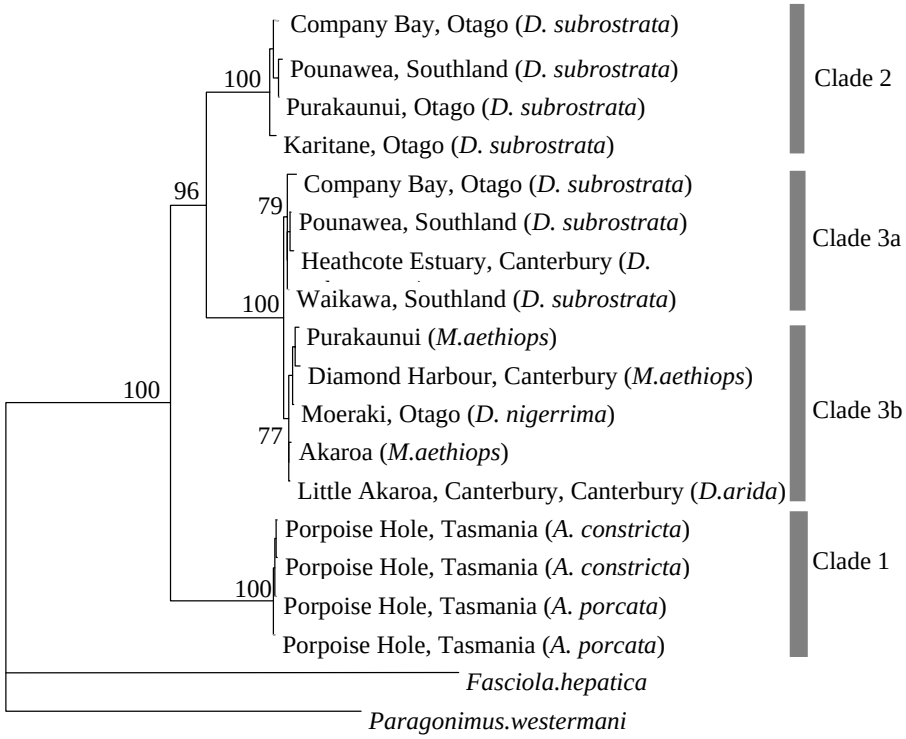
Figure 1: Phylogenetic relationships of fourteen topshell species collected from New Zealand, Australia and Chile inferred by neighbour joining analysis of approximately 2 kb of sequence data. The numbers associated with the branches represent NJ bootstrap values (10000 replicates). Two New Zealand molluscs, M. huttoni and C. tesselata, were used as outgroups.



Digenean infections occurred in 103 snails, representing four New Zealand (*M. aethiops*, *D. subrostrata*, *D. nigerrima* and *D. arida*) and two Australian species (*A. constricta* and *A. porcata*), with levels of infection in individual populations ranging from 0 to 17.5%. Sympatric species often exhibited large differences in levels of infection e.g. at Company Bay, near Dunedin, *D. subrostrata* and *M. aethiops* live in sympatry. The *D. subrostrata* population displayed an infection rate of 15% whereas the *M. aethiops* population was uninfected.

Phylogenetic analysis revealed that the single morphotype consisted of three well-supported clades (figure 2). The first comprised of digeneans that infected two Australian species. The second clade comprised of digeneans that were specific to *D. subrostrata*. The final clade consisted of digeneans isolated from all four infected New Zealand topshell species, those infecting *D. subrostrata* formed their own subgroup within this clade.

Figure 2: Phylogenetic relationships of digeneans isolated from six species of trochid topshells inferred by neighbour joining of approximately 0.75 kb of sequence data. The key to infected trochid populations is 'Sampling location (host species)'. The numbers associated with the branches represent NJ bootstrap values (10000 replicates). Two platyhelminths F.hepatica and P.westermani were used as outgroups.



4. DISCUSSION

Analysed in conjunction, the field study data documenting the geographic range and the molecular data gave some insight into topshell evolution and dispersal. Topshells originally dispersed from Australia to New Zealand. The species *A. concamerata* and *D. nigerrima* were then dispersed from New Zealand to Australia and Chile respectively, with the Chilean dispersal event occurring much more recently.

The large difference in infection rates between sympatric snail species indicated some degree of host specificity, which may be explained, in part, by the single parasite morphotype consisting of several genetically distinct groups, which are almost certainly separate species. Digeneans in each group showed differing degrees of host specificity, with both the phylogenetic relatedness and habitat preference of the host species appearing to affect the level of infection and degree of host sharing. Without the digenean genetic analyses, the interpretation of the field results would have been strikingly misleading with host specificity being underestimated i.e. one parasite species infecting six snail species in two countries.

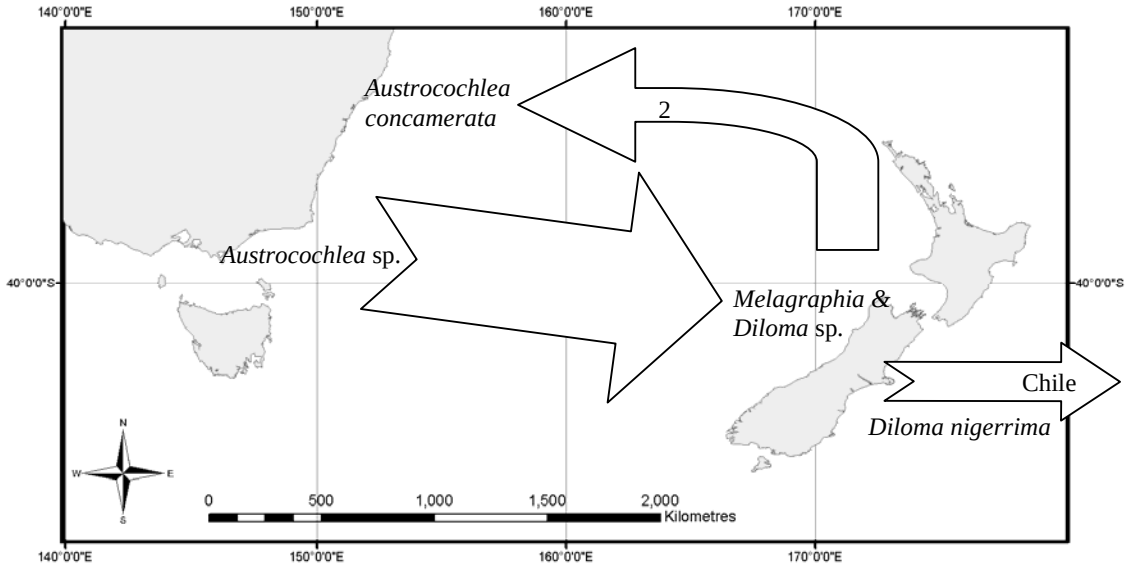


Figure 3: Dispersal of topshells

***Keywords and phrases*:** digenean, host specificity, phylogenetic tree, sympatric, topshell.

REFERENCES

Clark, W.C. (1958) A new cotylocercous cercaria from *Melagraphia aethiops* (GM) (Gastropoda). *Transactions of the Royal Society of New Zealand* 85, pp. 681-683.

Miller, A.A. and Poulin, R. (2001) Parasitism, movement and distribution of the snail *Diloma subrostrata* (Trochidae) in a soft-sediment intertidal zone. *Canadian Journal of Zoology* 79, pp. 2029-2035.

Paterson, A.M., Gray, R D. and Wallis, G.P. (1993) Parasites, petrels and penguins – does louse presence reflect seabird phylogeny? *International Journal for Parasitology* 23, pp. 515-526.

Swofford, D.L. (2002) PAUP* 4.0b1.0, Sinauer Associates, Sunderland, MA.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. and Higgins, P.G. (1997) The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* 24, pp. 4876-4882.

Walsh, P.S., Metzger, D.A. and Higuchi, R. (1991) Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *Biotechniques* 10, pp. 506-513.